TABLE I: Oligodeoxynucleotides

A. Thermodynamics and Kinetics

14-dC-S	ATG CAG CTA AGT CA
14-dC-A	TGA CTT AGC TGC AT
14-BrdC-S	ATG CAG CTA AGT CA
14-BrdC-A	TGA CTT AGC TGC AT
14-MedC-S	ATG CAG CTA AGT CA
14-MedC-A	TGA CTT AGC TGC AT
12-dC-S	CAT GCA GCC CCA
12-dC-A	TGG GGC TGC ATG
12-BrdC-S	CAT GCA GCC CCA
12-MedC-S	CAT GCA GCC CCA
16-dC-A	TGG GGC TGC ATG GCG T
16-BrdC-S	ACG CCA TGC AGC CCC A

Nomenclature: Oligodeoxynucleotide length - (dC, MedC, BrdC) - (S = sense; A = antisense).
C = 5'-Bromodeoxycytidine, C = 5'-Methyldeoxycytidine.

B. Linkers, Displacers and Targets

PstI

GAT GAT GAT GTG CAG CCA ATG CCC CAG GAG CCC T	P-D-MedC
GAT GAT GAT GTG CAG CCA AAG CCC CAG GAG CCC T	P-D-BrdC-E(10)
GAT GAT GAT GTG CAG CCA ATG CCC CAG GAG CCC A	P-D-BrdC-E(24)
GAT GAT GAT GTG CAG CCA ATG CCC CAG GAG CCC T	P-D-BrdC
G TAC CTA CTA CTA C	P-L-dC
*G CCA ATG CCC CAG GAG CCC T	P-T-D
AC GTC GGT TAC GGG GTC CTC GGG A	P-T-L

EcoRI

CCT CGA AGG AGC CTT CCA CAG CCG AAT TGT AGT AGT AGT AAG CT	E-D-BrdC
CA TCA TCA TCA T	E-L-dC
*CCT CGA AGG AGC CTT CCA CAG CCG	E-T-D
GGA GCT TCC TCG GAA GGT GTC GGC TTA A	E-T-L

XmaI (*SmaI*)

TCT CGG CTC ACT GCA ACG TCC GCC TCC CGG GTA GTA GTA GTA	X-D-BrdC
CAT CAT CAT CAT	X-L-dC
CC CAT CAT CAT CAT	Sm-L-dC
*TCT CGG CTC ACT GCA ACG TCC GCC TC	X-T-D
AGA GCC GAG TGA CGT TGC AGG CGG AGG GCC	X-T-L

SfiI

GTA GTA GTA GTA CCC CGG CCA CAC ACA CAC ACA CAC GA	S-D-BrdC
CAT CAT CAT CAT	S-L-dC

NotI

CTC TCT CTC TCT CTC TCT GCG GCC GTA GTA GTA GTA	N-D-BrdC
CAT CAT CAT CAT	N-L-dC

Nomenclature: Oligodeoxynucleotides are named *x-y-z* where:
x= restriction site (first letter only)
y= function (displacer, linker or target)
z= composition (BrdC, MedC, dC) or polarity (displacer or linker side)
E(N) = error at position N (a mismatch) if present

C. Plasmid pMS19 construction

EcoRI

AAT TGA GAT CTG GCC ACC CCG GGG ACA CAC ACA CAC ACG PMS-ES
CT CTA GAC CGG TGG GGC CGG TGT GTG TGT GTG TGC TTA A PMS-SE

HindIII

AGC TTG CTC TCT CTC TCT CTC TCT GCG GCC GCT TTT AGA TCT C PMS-NH
AC GAG AGA GAG AGA GAG AGA CGC CGG CGA AAA TCT AGA G TCG A PMS-HN

Nomenclature: Oligodeoxynucleotides are named *x-y* where:
x= function (PMS)
y= orientation in product (e.g. ES is *EcoRI* to *SfiI*)

D. Triplex-formation (at *SalI* in pMS19)

TCTCTCTCTCTCTCTCTC C AAGCTT GCATGC CTGCAG GTCGAC TCAATGATTCCC BT-D-MedC(1)-1
G AGTTACTAAGGG BT-L-dC-1

C AAGCTT GCATGC CTGCAG GTCGAC TCAATGATTCCC B0-D-MedC(1)-1
G AGTTACTAAGGG BT-L-dC-1

Nomenclature: Oligodeoxynucleotides are named *x-y-z* where x = BT if both branch migration and triplex formation are possible, x = B0 if only branch migration is possible; y = composition (dC, MedC or BrdC) in the branch migration region (1) and/or triplex forming region (2); z = laboratory index.

TABLE II: Bromodeoxycytidine and Methyldeoxycytidine Thermodynamics

Oligodeoxynucleotides	t_m in 1 M Na^+, C = 6 μM		
	pH 4	pH 7	pH 10
14–dC–A + 14–dC–S		57°	
14–dC–A + 14–BrdC–S		63°	
14–dC–S + 14–BrdC–A		62.5°	
14–BrdC–A + 14–BrdC–S		65°	
12–dC–A + 12–dC–S	60°	53.5°	50°
12–dC–A + 12–BrdC–S	70°	69.5°	54°
	pH 4.7	pH 7	pH 9.6
14–dC–A + 14–dC–S		57°	
14–dC–A + 14–MedC–S		60°	
14–dC–S + 14–MedC–A		59.5°	
14–MedC–A + 14–MedC–S		64.5°	
12–dC–A + 12–dC–S	50°	60°	47.5°
12–dC–A + 12–MedC–S	55°	67°	50.5°

TABLE III: Temperature Dependence of Displacement Rates with BrdC–Containing Displacers

A. Blunt ends

Oligodeoxynucleotides	Temp (°C)	Half–time (min) for displacement with 12–BrdC–S at: 101 μM	20 μM	4 μM
12–dC–S* (C = 0.25 μM) +	37	2	4–8	16–32
12–dC–A (C = 0.75 μM)	32	4–8	8–16	32
	27	4–8	32–64	128–256

B. Overhangs

Oligodeoxynucleotides	(°C)	Half–time (min) for displacement with 16–BrdC–S at: 3 μM	0.57 μM
12–dC–S* (C = 0.25 μM) +	37	<1	4–8
16–dC–A (C = 0.57 μM)	32	<1	16–32
	27	<1	2–4
* 5'–^{32}P–labeled	22	<1	<1

C. Effect of Linkers

Site	Overhang	G+C%	Half time for displacement (minutes) No linker	Linker
*Eco*RI	5′	0	60	6
*Pst*I	3′	50	8	< 1
*Xma*I	5′	100	8–16	< 1

CONDITIONS: Ligase buffer (pH = 7); 37°C; 10 μL per reaction.
Target: Kinased strand 10 ng, Unlabeled strand 30 ng.
Displacer: 150 ng; Linker (if present): large molar excess.
Displacer concentration = 1 μM.

TABLE IV: Linker Capture

	Displacer					
	34–BrdC–D		34–MedC–D		34–dC–D	
Fragment:	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
A	16	9	10	7	4	4
B	384	344	150	144	16	15
D	1*	1*	1*	1*	1*	1*

* by definition

TABLE V: Linker Capture with Mismatches

Displacer	Relative yield (Fragment B/Fragment A) Experimental	Calculated
P–D–BrdC	24	29
P–D–BrdC–E(10)	1	1
P–D–BrdC–E(24)	24	39

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